Experimental Confirmation of Bean Preference
For Callosbruchus Maculatus Females

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ABSTRACT

The purpose of this study is determine the existence or lack of bean preference in Callosobruchus maculatus. This is done by comparing the choice of female bean beetles when presented with a historic bean, the same species they hatched from, and a novel bean, one that has no ancestral hold on the female but may present a better habitat for her offspring. To test this we set up small habitats for each female with a choice of two beans. Then we recorded the number of eggs oviposited and on which bean. The results from our 2 trials showed a varying number of eggs laid and bean used. This means our hypothesis became null and we had to adopt an alternative hypothesis.

INTRODUCTION

The Callosobruchus maculatus bean beetles are indigenous to West Africa, and are believed to have been displaced though the movement of legume crops. The reproductive and life cycles of the bean beetle are fully dependent on the host bean the female chooses to deposit her egg on. Since there is no parental help to the young, reproductive success is tightly correlated with number and quality of eggs hatched. Body condition has an important role in the fitness of the C. masculatus. Their fitness is determined by the short yet crucial time of early life. Consequently, optimal and adverse conditions can strongly affect body mass and/or reproductive success. (Schade & Vamosi, 2012)

In a study that was done with mung beans, it was found that the average mung bean size is not ideal for a bean beetle to lay her eggs; however is a preferred food source for her offspring. Once inseminated, adult females will oviposit fertilized eggs on the external surface of a bean. Females often avoid laying multiple eggs on a single bean; instead, she will try to utilize as many beans as possible. (Mitchell, 1975) Once, the larva hatches from the egg, it burrows through the seed coat and into the bean's endosperm. The larva feeds on the bean's endosperm and embryo, while it undergoes a series of molts. Finally, just before pupation it burrows to a position just underneath the seed coat. The adult that results from pupation chews through the seed coat and emerges from the bean. The adults are fully mature 24 to 36 hours, after emergence. Neither male nor female adults require food or water during their short adult lifetime 10-14 days. (Blumer, Beck, Brown & Downhower)

Since the choice of prey bean is the most critical choice a female makes for her offspring,
she studies certain features. Factors that the female considers include: quantity and/or quality, plant morphology, natural enemies, competition, and chemicals all of which serve as cues to identify suitable hosts for oviposition. (Ballhorn & Lieberei, 2006) Some of the beans have a seed coat with a toxin that will cause larvae to be too weak to survive. Once ingested this toxin affects how the larvae penetrate the seed coat, ultimately causing them to die of starvation. (Jenzen 1977) If typical host beans are unavailable to the female, they may 'dump' their eggs on the next available bean, even though it may be an unsuitable host. This act can cause larval mortality and even reduce maternal life span. (Messima & Fox, 2011) Our hypothesis is that since bean beetles have a choice of which bean to oviposit their eggs on, when presented with two equally viable beans, one historic- the bean the mother was hatched form- and one novel, they will consistently choose the historic bean to grow their offspring.

METHODS

Our experiment was conducted in March 2014 at the University of Wisconsin- Whitewater. We wanted to determine if female bean beetles showed any preference as to which bean she laid her eggs on. In order to test our hypothesis the Mung, Vigna radiate, and Adzuki, Vigna angularis, beans were chosen to be the novel beans in our experiment. All female bean beetles used for this experiment were hatched on Black Eyed Peas, Vigna unguiculata. The Adzuki and Black Eyed Peas are relatively the same size with an average diameter of 8-10 millimeters each. Mung beans are smaller than the Adzuki and Black Eyed Peas with a diameter of 5 millimeters. We discarded any beans, of any species, that had missing portions or appeared to be disfigured when compared to the majority of the others.

We set up a total of six petri dishes, 40mm x 12mm. All six petri dishes had a black eyed pea placed on one side. Three of the petri dishes had an Adzuki bean, three had a Mung bean placed on the other side. Each petri dish had a total of two beans in them. In order to maintain a well-lit environment, we placed a light bulb, 100 watt, 10 inches above our petri dishes, so the heat would not be a factor in the female's decision, and made sure that the light was on for the duration of the study, seven days. The light tricked the female's into thinking the day was longer than it was hence driving her to lay her eggs.

We checked each petri dish every other day and recorded our findings: if the female had laid eggs, we recorded which bean(s) she had laid on, and how many eggs were present. If eggs were laid before the end of the seven day period and the beetle died we included all counts in our findings. If a beetle died without laying any eggs we did not factor those results into our findings. We only included the petri dishes that had eggs, if no eggs were present on any of the beans in a petri dish at the end of the seven days the beans and the female were discarded. After the first full experiment we repeated the whole process for more accurate results.
RESULTS

Our results for each trial were somewhat what we had expected, one trial supported our hypothesis and the other supported our alternative hypothesis. In order to determine if this difference is significant we took the total sum of the Observed minus the Expected then squared this value, then we divided that number by the number of eggs that we expected (Fig. 1 and 2).

For the first trial, Black Eyed Peas (BEP) vs Mung, there were a total of 28 eggs laid (Fig. 3). We observed 17 eggs on the BEP and 11 eggs on the Mung bean. We use the $\chi^2$ distribution chart where $P = 0.05$ along with our degrees of freedom (df), for both cases $df = 1$, to determine if this difference is significant enough to accept or reject our null hypothesis at the 95% confidence level. The results of this test show us that our $\chi^2 = 1.285$, our $\chi^2$ at 1 degree freedom is 3.84, according to the chart. (Fig. 1) There is not enough difference between the female eggs laid on BEP vs Mung to say that a historical preference was shown. We can reject our null hypothesis and support the alternative one, because our $\chi^2$ is less than 3.84.

For the second trial, BEP vs Adzuki, there were a total of 11 eggs laid (Fig 4). We observed 2 eggs on the BEP and 9 eggs on the Adzuki bean. Using the same methods as mentioned above, we found our $\chi^2 = 4.46$ (Fig. 2). This value is greater than 3.84 and tells us that some significant preference was shown by the female beetle. However, since more eggs were laid on the Adzuki bean than the BEP, we will still reject our null hypothesis and support our alternative one. This tells us that the female bean beetle showed some preference for the bean that she laid her eggs on, but that this most likely didn’t have to do with a historical preference.

Figure 1

Trial 1 BEP vs Mung had an additional Mung and BEP egg laid making our total 17 eggs laid on BEP between both trials and 11 eggs laid on Mung beans.
Figure 2

Trial 2 BEP vs Adzuki; 2 eggs laid on BEP between both trials and 9 eggs laid on Adzuki beans.

Figure 3

Eggs Laid between BEP vs Mung (#1 - #3) / Eggs Laid between BEP vs Adzuki (#4 - #5)

Figure 4

Eggs Laid between BEP vs Mung (#1 - #3) / Eggs Laid between BEP vs Adzuki (#4 - #5)
DISCUSSION

Two trials were conducted in this experiment. Trial one showed no significant indication that the ancestral bean was preferred. However, in trial two there was a notable difference in the bean preference. We compared the home bean, Black Eyed Pea, with either the Mung or Adzuki bean. In another study, Kawecki and Mery (2003), found that bean preference is significant amongst female bean beetles. However in our experiment females chose to lay more eggs on non-ancestral than ancestral beans, given a choice. There was no significant difference between the three choices of beans, per relative trial, and whether the female laying preference was affected. This outcome did not support our hypothesis. Hence, the alternative hypothesis was supported. One explanation for this is the female’s behavior is that the beans that they laid eggs on were superior over the ancestral bean. Another explanation as to why the ancestral bean was not chosen is there was an natural selection progression to a different bean choice.

There are some errors that became apparent during each trial that we would try to control if we were to do this experiment again. The main issue was the survival of the female bean beetles. Most of the female beetles used in these trials did not survive and as a result we were not able to gather any recordable data. Even though we rejected our null hypothesis, this could be attributed to this issue. Due to the high mortality rate of the female beetles our overall data was greatly affected and resulted in diminished recordable data.

If we were to redo this experiment, the beetles chosen should be young. Their life cycle is only two weeks, so choosing beetles in their first few days may raise our mortality rate. Also there should be more effort put into the well-being of each beetle; try to create an environment that is more suitable for beetle survival. Whether this means the addition of food/water, a day/night cycled environment, or possibly using our alternative hypothesis as our null hypothesis in a future experiment, by ensuring beetle survival we will get more accurate results in return. We would also run our trials more that twice, continuing trials will give us more usable data.
BIBLIOGRAPHY


